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3 INDEPENDENT SIZING OF STR GEL IMAGES

3.1 INTRODUCTION

Each casework examiner and DNA Data Bank Analyst utilizes the STaRCall allele calling software to assign an allele designation for each sample band at each locus, followed by another qualified individual independently determining the allele designation using the STaRCall software. The Forensic Laboratory Specialist (FLS) will be deemed qualified to perform this independent calling of alleles after the successful completion of the following training program, estimated to take approximately 1½ months. The training consists of six training sets and is designed to ensure that the FLS has sufficient time to become familiar with the FMBIO Fluorescent Image Analysis System, has a good understanding of how the software is used, can accurately call alleles using the specified procedures, and can properly document the results. Identical training sets for each laboratory will be provided by the Section Chief to be maintained by that laboratory. Periodically, new training sets may be distributed.

3.2 GOALS

- 3.2.1 To become familiar with the FMBIO II/FMBIO III Fluorescent Image Analysis System software used to assign base pair values to the alleles in the PowerPlex[®] 16 BIO System.
- 3.2.2 To become familiar with the STaRCall software used to assign allele designations.
- 3.2.3 To become familiar with the visual interpretation of the typing gel.
- 3.2.4 To understand the use of controls and the internal lane standard.
- 3.2.5 To understand the problems that may be encountered interpreting allele designations.
- 3.2.6 To accurately and independently assign base pair values to the alleles in the PowerPlex[®] 16 BIO System and to accurately complete the appropriate documentation for the casework examiner or Data Bank analyst.

3.3 TASKS

- 3.3.1 Become familiar with the FMBIO II/FMBIO III software. Refer to the <u>Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual Section III, Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex 16 BIO System for the procedure.</u>
- 3.3.2 Training Set 1 (Week 1):
 - 3.3.2.1 In order to ensure a thorough understanding of how to use the FMBIO Fluorescent Image Analysis System, the training coordinator will provide a demonstration using the FMBIO and STaRCall software programs
 - 3.3.2.1.1 The training coordinator will demonstrate how to perform a gray scale correction, a color separation, and how to mark each sample and allelic ladder band using the FMBIO software.

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- 3.3.2.1.2 The training coordinator will explain the purpose of the allelic ladder, Internal Lane Standard, the 9947A known standard (Positive Amplification Control), and the Negative Amplification Control and how these are used during the sizing of the gel image.
- 3.3.2.1.3 After all bands have been marked, the training coordinator will demonstrate how to use the STaRCall allele calling software.
- 3.3.2.1.4 The training coordinator will provide a list of the known standard values for the 9947A Cell Line to the FLS, so that he/she can ensure that the gel has run properly. This list will be used by the FLS to compare the known standard values for the 9947A Cell Line (PowrPlex® 16 BIO gels) to the values on each gel to ensure that the correct allele designations have been obtained.
- 3.3.2.2 After a clear understanding is developed of how the FMBIO and STaRCall software programs are used and the purpose of each control (i.e., 9947A Cell Line and the Negative Amplification Control), the training coordinator will provide training set 1 consisting of three PowerPlex[®] 16 BIO gel images (Training Set 1) to be sized.
- 3.3.2.3 Size all three gel images, print a copy of the gel images and the associated STaRCall and Landscape sheets and Lookup Tables and provide these to the training coordinator in the training notebook described in Section 1 of this manual.
 - 3.3.2.3.1 The training coordinator will verify that all of the alleles were assigned correctly when compared to the target allele designations and review the results and the associated documentation with the FLS.
 - 3.3.2.3.2 If any of the allele designations were assigned incorrectly, the training coordinator will conduct an investigation to determine the extent of the problem, to identify why the problem occurred, and what steps will be implemented to prevent the problem from recurring. All problems identified at this stage of training should be corrected before training continues.
- 3.3.3 Training Set 2 (Week 2):
 - 3.3.3.1 After it has been determined that all results from Training Set 1 are correct, the training coordinator will assign three PowerPlex[®] 16 BIO gel images (Training Set 2) to be sized.
 - 3.3.3.2 Size all three gel images, print a copy of the gel images and the associated STaRCall and Landscape sheets and Lookup Tables and provide these to the training coordinator to compare to the target values.
 - 3.3.3.2.1 If all results are correct, the training coordinator will review the results and the associated documentation with the FLS.
 - 3.3.3.2.2 If any of the allele designations were assigned incorrectly, the training coordinator will conduct an investigation to determine the extent of the problem, to identify why the problem occurred, and what steps will be

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implemented to prevent the problem from recurring. This investigation will be completed and reported to the supervisor of the Forensic Biology Section. The supervisor will review the results of the investigation and may consult with the Section Chief before the training continues.

3.3.4 Training Set 3 (Week 3):

- 3.3.4.1 After it has been determined that all results from Training Set 2 are correct, the training coordinator will assign three additional PowerPlex[®] 16 BIO gel images (Training Set 3) to be sized.
- 3.3.4.2 Size all three gel images, print a copy of the gel images and the associated STaRCall and Landscape sheets and Lookup Tables and provide these to the training coordinator to compare to the target values.
 - 3.3.4.2.1 If all results are correct, the training coordinator will review the results and the associated documentation with the FLS.
 - 3.3.4.2.2 If any of the allele designations were assigned incorrectly, the training coordinator will conduct an investigation to determine the extent of the problem, to identify why the problem occurred, and what steps will be implemented to prevent the problem from recurring. This investigation will be completed and reported to the supervisor. The supervisor will review the results of the investigation and may consult with the Section Chief before the training continues.

3.3.5 Training Set 4 (Week 4):

- 3.3.5.1 After it has been determined that all results from Training Set 3 are correct, the training coordinator will assign three PowerPlex[®] 16 BIO gel images (Training Set 4) to be sized.
- 3.3.5.2 Size all three gel images, print a copy of the gel images and the associated STaRCall and Landscape sheets and Lookup Tables and provide these to the training coordinator to compare to the target values.
 - 3.3.5.2.1 If all results are correct, the training coordinator will review the results and the associated documentation with the FLS.
 - 3.3.5.2.2 If any of the allele designations were assigned incorrectly, the training coordinator will conduct an investigation to determine the extent of the problem, to identify why the problem occurred, and what steps will be implemented to prevent the problem from recurring. This investigation will be completed and reported to the supervisor. The supervisor will review the results of the investigation and may consult with the Section Chief before the training continues.

3.3.6 Training Set 5 (Week 5):

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3.3.6.1 After it has been determined that all results from Training Set 4 are correct, the training coordinator will assign three PowerPlex[®] 16 BIO gel images (Training Set 5) to be sized.

- 3.3.6.2 Size all three gel images, print a copy of the gel images and the associated STaRCall and Landscape sheets and Lookup Tables and provide these to the training coordinator to compare to the target values.
 - 3.3.6.2.1 If all results are correct, the training coordinator will review the results and the associated documentation with the FLS.
 - 3.3.6.2.2 If any of the allele designations were assigned incorrectly, the training coordinator will conduct an investigation to determine the extent of the problem, to identify why the problem occurred, and what steps will be implemented to prevent the problem from recurring. This investigation will be completed and reported to the supervisor. The supervisor will review the results of the investigation and may consult with the Section Chief before the training continues.

3.3.7 Training Set 6 (Week 6):

- 3.3.7.1 After it has been determined that all results from Training Set 5 are correct, the training coordinator will assign three PowerPlex[®] 16 BIO gel images (Training Set 6) to be sized.
- 3.3.7.2 Size all three gel images, print a copy of the gel images and the associated STaRCall and Landscape sheets and Lookup Tables and provide these to the training coordinator to compare to the target values.
 - 3.3.7.2.1 If all results are correct, the training coordinator will review the results and the associated documentation with the FLS.
 - 3.3.7.2.2 If any of the allele designations were assigned incorrectly, the training coordinator will conduct an investigation to determine the extent of the problem, to identify why the problem occurred, and what steps will be implemented to prevent the problem from recurring. This investigation will be completed and reported to the supervisor. The supervisor will review the results of the investigation and may consult with the Section Chief before the training continues.

NOTE:

Additional images may be provided by the training coordinator (provided by the supervisor to the training coordinator with accompanying target values) at any time during the training period to satisfy the requirement of successfully sizing a minimum of 150 individual samples and eighteen 9947A Cell Line controls, as appropriate. If problems continue to occur throughout the Forensic Laboratory Specialist's training, a re-evaluation of the training will be conducted and appropriate action taken. The supervisor will consult with the Section Chief about ongoing problems with the FLS obtaining the correct results.

3.3.8 Competency Test:

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- 3.3.8.1 Upon successful completion of sizing a minimum of 150 individual samples and eighteen 9947A Cell Line controls (as appropriate), the training coordinator will provide a final competency test. This test, previously approved by the supervisor, will consist of moderately challenging PowerPlex[®] 16 BIO gel images from a previously analyzed case containing at least known samples from a victim and suspect, two items of evidence or one item differentially extracted, and all appropriate controls.
- 3.3.8.2 Size the competency test gel images, print a copy of the gel images and the associated STaRCall and Landscape sheets and Lookup Tables and provide these to the training coordinator to compare to the previously obtained results.
- 3.3.8.3 If any of the allele designations were assigned incorrectly, the trainer will conduct an investigation to determine the extent of the problem, to identify why the problem occurred, and what steps will be implemented to prevent the problem from recurring. This investigation will be completed and reported to the supervisor. The supervisor will review the results of the investigation and consult with the Section Chief before any further action is taken.

3.4 TRAINING EVALUATION

- 3.4.1 Evaluation of documentation skills by the training coordinator.
- 3.4.2 The FLS should be able to successfully complete the independent sizing of a minimum of 150 individual samples, eighteen 9947A Cell Line controls (as appropriate), and a competency test. This will be evaluated and monitored throughout the training.
- 3.4.3 Completion of the checklist by the training coordinator. The original checklist signed and dated by the training coordinator will be forwarded by the supervisor to the Laboratory Director or their designee in accordance with the Department Quality Manual.

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CHECKLIST FOR INDEPENDENT SIZING OF STR GEL IMAGES

Trainee has su	ccessfully sized all typing gel images using the STaRCall software.
Date:	Training Coordinator:
Comments:	
Trainee has su necessary.	ccessfully demonstrated the ability to perform gray scale corrections and color separations w
Date:	Training Coordinator:
Comments:	
T	
i rainee under	stands the purpose of each of the work sheets generated using the STaRCall software.
Date:	
Date:	
Date: Comments: Trainee has de II/FMBIO III	Training Coordinator: emonstrated his/her ability to visually interpret the scanned images generated by the FMBIO Fluorescent Image Analysis System.
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7. Notebook is	organized and complete.			
	Training Coordinator:			
Comments:				
Recommended by:		Date:		
•	Training Coordinator			
Qualified by:	g .	Date:		
	Supervisor			
				♦END
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